REMARKS

Initially, Applicants thank the Examiner for withdrawing the previous rejections of record as noted on Page 2 of the Official Office Action.

Applicants again note that a Form PTO-948, indicating the Draftsperson's approval was not included with the Office Action, and Applicants respectfully request that Examiner include this paper in the next communication from the U.S.P.T.O.

Applicants wish to thank the Examiner for the courtesy extended to Applicants representative Sean Ryder during a telephonic interview on August 5, 2003. During the interview, the patentability of claim 5 was discussed, as well as amendments to overcome the rejection of record of the same. In particular, the Examiner noted that for claims such as claim 5, a representative number of examples of mutant DNA sequences, or a teaching in the specification pointing to a particular region which can be mutated is required for enablement. Applicants representative Sean Ryder raised the issue of knowledge of one of ordinary skill in the art and the degeneracy of the genetic code, as reiterated below.

Reconsideration and withdrawal of the restriction requirement and rejections of record are respectfully requested.

Summary of Status of Amendments and Office Action

In the present amendment, claim 5 is amended. Claims 1 and 2 have been withdrawn from consideration in light of the Restriction Requirement dated November 1, 2001. Therefore, claims 3-6 remain pending in the application with claims 3-6 being independent.

In the Office Action, claim 5 is rejected under 35 U.S.C. § 112, first paragraph, as not being enabled.

Explanation and Support for Amendments

Applicant submits that each of the foregoing amendments is fully supported by the specification.

Response to Restriction Requirement

Applicants again note that the Examiner has made the Restriction Requirement final. However, Applicants are allowing the non-elected claims to remain pending until such time as an indication of allowability with respect to all remaining claims is received in this application.

Response to § 112, first paragraph Rejections

Claim 5 is rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a full length DNA set forth in SEQ ID NO:2 encoding the protein of SEQ ID NO:1, allegedly does not reasonably provide enablement for the DNA having a nucleotide sequence with one or more nucleotide sequence with one or more nucleotide substitutions relative to the nucleotide sequence of SEQ ID NO:2, which encodes a protein of SEQ ID NO:1. The Office action asserts that the specification does not enable one of ordinary skill in the art to make/use the invention commensurate in scope with claim 5.

In response, Applicants note that the current amendment to claim 5 would more clearly set forth the invention and point out that the claimed DNA would still have the same number of nucleotides and codons. Further, as amended, claim 5 more clearly sets forth that the protein sequence and function are not changed by the codon substitutions. Applicants respectfully submit that, as amended, one of ordinary skill in the art, having the DNA sequence set forth in SEQ ID NO:2 and using a DNA code table, would be able to practice the claimed invention by substituting codons in the sequence of SEQ ID NO:2 such that the final protein encoded would still have the amino acid sequence set forth in SEQ ID NO:1 and the same function as the protein encoded by the wild-type DNA sequence. Applicants note that the Office Action stated that this argument has been considered and rejected because the claimed subject matter would require one of ordinary skill in the art to "generate an unspecified number of mutants ." However, it is respectfully submitted that one of ordinary skill in the art would not need to do this. In this regard, consideration of the

following is respectfully requested.

Initially, Applicants note that the genetic code is degenerate, and each amino acid (with the exception of tryptophan and methionine) is encoded by more than one different codon. Thus, for instance, one of ordinary skill in the art would recognize that the DNA sequence TTA and CTG both encode leucine and are therefore interchangeable when designing a gene encoding a specific amino acid sequence including leucine. Applicants respectfully direct the Examiner's attention to the enclosed pages 172-174 of Genes V (1994) which discuss the degeneracy of the genetic code. This document notes that the degeneracy of the code "minimizes the effects of mutations" on the encoded protein and "increases the possibility that a single random base change will result in no amino acid substitution. . . [f]or example, a mutation of CUC to CUG has no effect." Genes V, page 173. This reference evidences that as far back as 1994, codon substitution was recognized in the art as having no effect if the codons encode the same amino acid. Therefore, Applicants do not understand the Office Actions' assertion of unpredictability in the art with regard to the substitution. One of ordinary skill in the art would simply need to determine which amino acid the wild-type codon encodes, look up the amino acid in a codon table, and know, with 100% certainty, that a substituted codon encoding the same amino acid will not change the encoded sequence of the protein. If the amino acid sequence is not changed, then neither is the function of the protein itself changed.

The Office Action states that the breadth of the claims was enlarged by the previous amendment. This statement is respectfully traversed. As amended, claim 5 has the same scope as did the previous claim 5, which in turn, contrary to the assertions of the Office Action, had the same

scope as did the initial claim 5. Claim 5 has always contained the limitation that irrespective of the mutations to the DNA sequence, that sequence still encoded the amino acid sequence set forth in SEQ ID NO:1. Thus, it is not seen how the claim was broadened by setting forth the types of mutations even more clearly and explicitly stating the scientifically obvious fact that the function of the protein remains the same.

Further, the Office Action asserts that the specification "provides only a generic description of how a variety of mutants can be generated, [but] no specific guidance is provided on the generation of the mutants or fragments that demonstrate the biological activity of the full length protein or DNA sequences." However, a generic description is all that one of skill in the art requires, as through use of a codon table, one of ordinary skill in the art would know how to mutate the nucleotide sequence such that the resulting codons encode the exact same amino acid sequence. Further, it is axiomatic in science that the function of a protein is defined by its amino acid sequence. If the amino acid sequence does not change, obviously the function of the full length protein does not change. Finally, in contrast to the Office Action, the claims doe not encompass fragments of the Rim2 DNA.

Additionally, in contrast to the assertions in the rejections, the Examiner is respectfully reminded that the burden is not on Applicants to establish that the claims are enabled, but is on the Examiner to support an enablement rejection using technical arguments. See, for example, "Training Materials for Examining Patent Applications with Respect to 35 U.S.C. Section 112, First Paragraph -- Enablement Chemical/Biotechnical Applications" and In re Marzocchi, 439 F.2d 220, 224, 169

USPQ 367, 369 (CCPA 1971).

In particular, in Marzocchi, in reversing the rejection, the Court noted that the Patent Office should not be concerned with the breadth of the claims per se and that the burden of showing lack of enablement is on the Patent Office:

> Turning specifically to the objections noted by the board as indicated above, it appears that these comments indicated nothing more than a concern over the breadth of the disputed term . . . . The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion.
>
> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of §112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. . . .
>
> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis [lack of enablement] is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement.

Id. at 369-70 (emphasis in original). Therefore, the burden of showing lack of enablement is on the Patent Office.

In fact, the Board of Appeals and Interferences relied upon Marzocchi in a case which is similar to the present case. See Ex parte Reese, 40 USPQ2d 1221 (Bd. Pat. App. & Int. 1996). The Board, however, stated that Ex parte Reese was not written for publication, and that the case was not binding precedent of the Board. Even though Ex parte Reese is not binding precedent, its application

of the law is informative.

In Ex parte Reese, the examiner rejected claims reciting a chemical formula "wherein R^1 represents C_1 - C_4 alkyl, R is the deoxy residue of a protected carbohydrate compound, R being different from R' [sic R^1?], and Ar is a monocyclic aryl group having an electron-withdrawing substituent which renders the group acid-labile." The examiner believed that the scope of enablement provided in the applicant's specification was not commensurate with the scope of protection sought. The examiner argued that the claims were broad and that the specification required more working examples.

In reversing the Examiner's rejection, the Board reasoned that an enablement rejection cannot be based upon subjective opinions, but must be based upon evidence or sound scientific reasoning. Id. at 1222. The Board also stated that recent case law puts the burden on the examiner. Id. at 1223.

In the present case, Applicants have rebutted any case the Office Action may have made to support the rejection of claim 5, and the rejection should be withdrawn (assuming, *arguendo*, that a case had been made). In view of the above, Applicants respectfully request that the rejection be withdrawn, and claim 5 be allowed. Applicants respectfully submit that this amendment is a clarifying amendment which does not narrow the scope of the claimed invention.

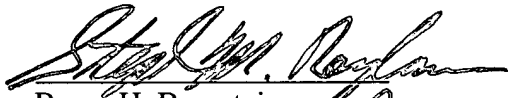
In accordance with M.P.E.P.§ 609C(3), the document cited above in support of Applicants' remarks is being submitted as evidence directed to an issue raised in the mentioned Official Action, and no additional fee or Certification pursuant to 37 C.F.R. §§ 1.97 and 1.98, or citation on a FORM PTO-1449 is believed to be necessary.

CONCLUSION

For the reasons advanced above, Applicants respectfully submit that all pending claims patentably define Applicants' invention. Allowance of the application with an early mailing date of the Notices of Allowance and Allowability is therefore respectfully requested.

If any issues remain which can be expeditiously resolved by a telephone conference, or if the Examiner has any questions concerning this matter or the application, the Examiner is respectfully invited to contact the undersigned at the below-listed telephone number.

Respectfully submitted,
Sususmu SEINO et al.



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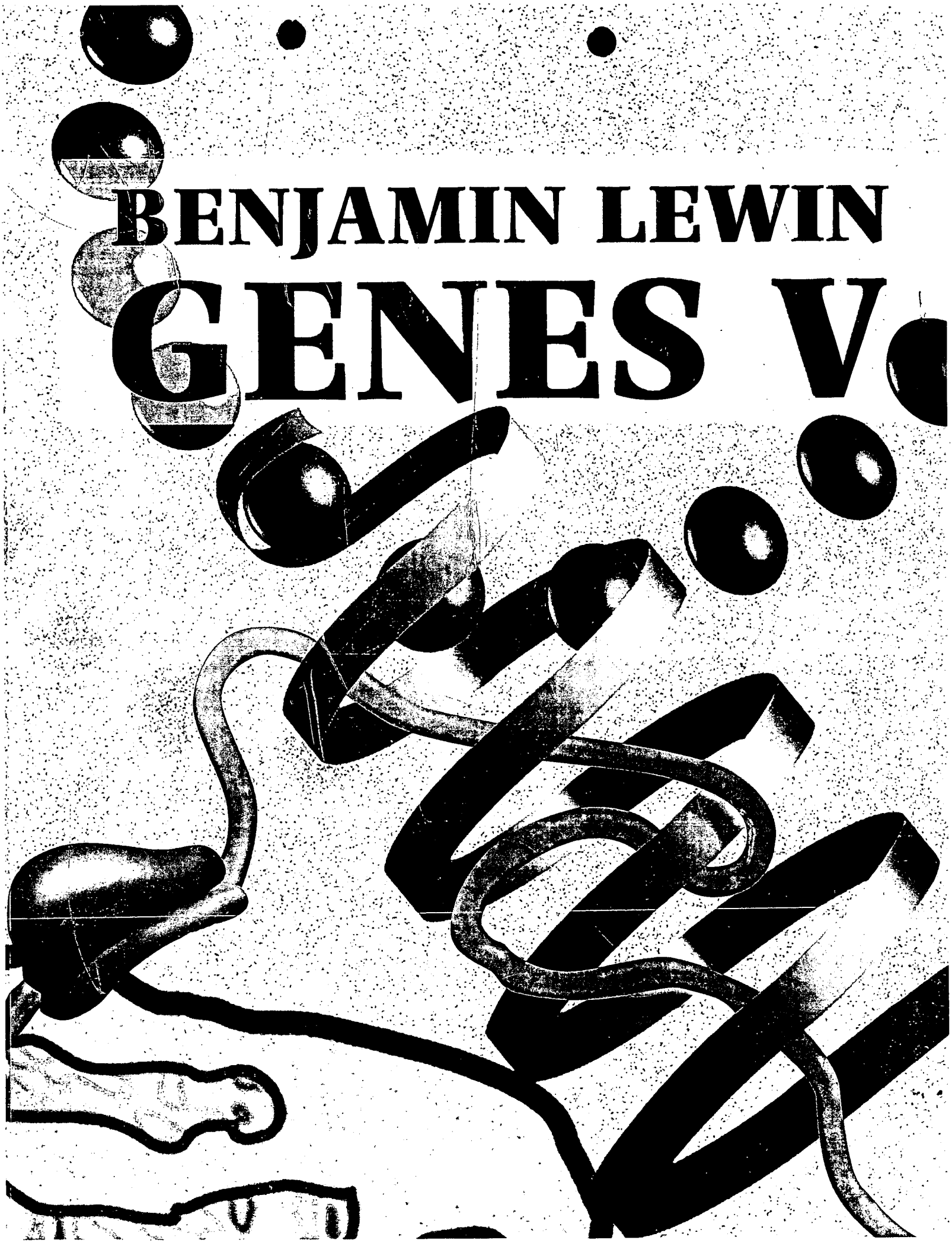

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different bases, were used by Khorana; they allowed the meaning of about half of the 64 codons to be assigned.

◆ The **ribosome-binding assay** for making codon assignments was developed by Nirenberg and Leder in 1964. A trinucleotide can be used to mimic a codon, by causing the corresponding aminoacyl-tRNA to bind to a ribosome. A triple complex of trinucleotide•aminoacyl-tRNA•ribosome can be isolated by taking advantage of the ability of ribosomes to bind to nitrocellulose filters. The aminoacyl-tRNA itself does not bind to such filters, but is retained as part of the triple complex. Its retention is detected by means of a radioactive label in the amino acid component. Then the meaning of each trinucleotide can be determined by testing which one of 20 labeled aminoacyl-tRNA preparations is retained on the filter.

The two techniques together assigned meaning to all of the codons that represent amino acids. Since then, the sequencing of DNA has made it possible to compare corresponding nucleotide and amino acid sequences directly. *The sequence of the coding strand of DNA, read in the direction from 5′ to 3′, consists of triplets corresponding to the amino acid sequence of the protein read from N-terminus to C-terminus.* The entire genetic code has been confirmed in overwhelming detail from such analysis.

The code is summarized in **Figure 7.9**. A striking feature is its **degeneracy**: almost every amino acid

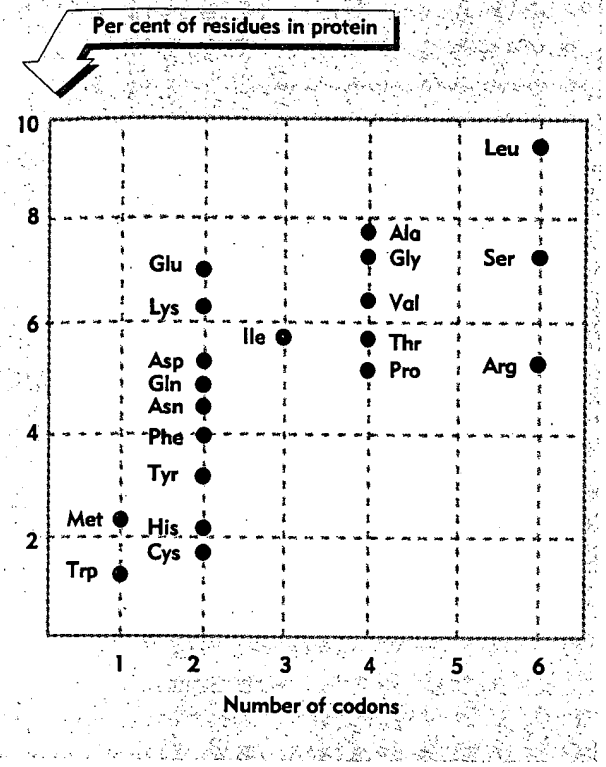
Figure 7.9

All the triplet codons have meaning: 61 represent amino acids, and 3 cause termination (TERM).

First base ↓ / Second base →	U	C	A	G
U	UUU, UUC: Phe UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr UAA, UAG: TERM	UGU, UGC: Cys UGA: TERM UGG: Trp
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg
A	AUU, AUC, AUA: Ile AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn AAA, AAG: Lys	AGU, AGC: Ser AGA, AGG: Arg
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly

Figure 7.10

The number of codons for each amino acid does not correlate with its frequency of use in proteins.



is represented by several codons. The only exceptions are methionine and tryptophan. Codons that have the same meaning are called **synonyms.** Figure 7.10 plots the number of codons representing each amino acid against the frequency with which the amino acid is used in proteins (in *E. coli*). Although there is a tendency for amino acids that are more common to be represented by more codons, this is slight, and therefore it does not seem that the genetic code has been optimized with regard to the utilization of amino acids.

Codons representing the same or related amino acids tend to be similar in sequence. Often the base in the third position of a codon is not significant, because the four codons differing only in the third base represent the same amino acid. Sometimes a distinction is made only between a purine versus a pyrimidine in this position. The reduced specificity at the last position is known as **third-base degeneracy.**

The tendency for similar amino acids to be represented by related codons minimizes the effects of mutations. It increases the probability that a single random base change will result in no amino acid substitution or in one involving amino acids of similar character. For example, a mutation of CUC to CUG has no effect, since both codons represent leucine; a mutation of CUU to AUU results in replacement of leucine with isoleucine, a closely related amino acid.

Three codons (UAA, UAG and UGA) do not represent amino acids. They are used specifically to terminate protein synthesis; one of these **stop codons** marks the end of every gene.

Is the genetic code the same in all living organisms?

Comparisons of DNA sequences with the corresponding protein sequences reveal that the *identical set of codon assignments is used in bacteria and in eukaryotic cytoplasm.* As a result, mRNA from one species usually can be translated correctly *in vitro* or *in vivo* by the protein synthetic apparatus of another species. Thus the codons used in the mRNA of one species have the same meaning for the ribosomes and tRNAs of other species.

The universality of the code argues that it must have been established very early in evolution. Originally there may have been a stereochemical relationship between amino acids and the codons representing them. Then the system now used for protein synthesis evolved by selection for features such as greater efficiency or accuracy.

Perhaps the code started in a primitive form in which a small number of codons were used to represent comparatively few amino acids, possibly even with one codon corresponding to any member of a group of amino acids. More precise codon meanings and additional amino acids could have been introduced later. One possibility is that at first only two of the three bases in each codon were used; discrimination at the third position could have evolved later.

Evolution of the code could have become 'frozen'

at a point at which the system had become so complex that any changes in codon meaning would disrupt existing proteins by substituting amino acids. Its universality implies that this must have happened at such an early stage that all living organisms are descended from a single pool of primitive cells in which this occurred.

Exceptions to the universal genetic code are rare. Changes in meaning in the principal genome of a species usually concern the termination codons. For example, in a mycoplasma, UGA codes for tryptophan; and in certain species of the ciliates *Tetrahymena* and *Paramecium*, UAA and UAG code for glutamine.

Systematic alterations of the code have occurred only in mitochondrial DNA. Such changes may have been possible because the mitochondrion is a relatively closed system, concerned with the synthesis of a few specific proteins. The majority of these changes affect initiation and termination, but other substitutions of meaning are also found. We discuss changes in the genetic code in Chapter 8, in the context of the interaction between aminoacyl-tRNA and messenger RNA.

The ribosomal sites of action

Synthesis of proteins involves an assembly line in which the ribosomes proceed inexorably along the messenger, bringing in the aminoacyl-tRNAs that provide the actual building blocks of the protein product. The ribosome itself constitutes a small mobile factory, in which a compact package of proteins and rRNAs forms several active centers able to undertake various catalytic activities. Different sets of accessory factors assist the ribosome in each of the three stages of protein synthesis: initiation, elongation, and termination. Energy for ribosome movement is provided by hydrolysis of GTP.

◇ **Initiation** involves the reactions that precede formation of the peptide bond between the first two amino acids of the protein. It requires the ribosome to bind to the mRNA, forming an initiation complex that contains the first aminoacyl-tRNA. This is a relatively slow step in protein synthesis, and usually determines the rate at which an mRNA is translated.

◇ **Elongation** includes all the reactions from synthesis of the first peptide bond to addition of the last amino acid. Amino acids are added to the chain one at a time; the addition of an amino acid is the most rapid step in protein synthesis.

◇ **Termination** encompasses the steps that are needed to release the completed polypeptide chain; at the same time, the ribosome dissociates from the mRNA.

Protein synthesis overall is a rapid process, although the rate depends strongly on temperature. In bacteria at 37°C, ~15 amino acids are added to a growing polypeptide chain every second. So it takes only ~20 seconds to synthesize an average protein of 300 amino acids. In eukaryotes, the rate of protein synthesis is slower; in red blood cells at 37°C, elongation typically sees ~2 amino acids added to the chain per second.

Most of the experiments to define the stages of protein synthesis have been performed with *in vitro* systems, consisting of ribosomes, aminoacyl-tRNAs, other enzymatic factors, and an energy source. In these systems, the rate of protein synthesis is slower by an order of magnitude than the rate *in vivo*.

Messenger RNA is associated with the small subunit, ~30 bases of the mRNA being bound at any time. But only two molecules of tRNA are involved in peptide bond synthesis at any moment. So polypeptide elongation involves reactions taking place at just two of the (roughly) ten codons covered by the ribosome.